Synthesis and Evaluation of Multisubstrate Inhibitors of an Oncogene-Encoded Tyrosine-Specific Protein Kinase. 1

Carolyn H. Kruse,† Kenneth G. Holden,*,† M. Lynn Pritchard,‡ John A. Feild,‡ David J. Rieman,‡ Russell G. Greig,‡ and George Poste‡

Departments of Medicinal Chemistry and Cell Biology, Smith Kline & French Laboratories, P.O. Box 7929, Philadelphia, Pennsylvania 19101. *Received August 18, 1987*

The synthesis and testing of potential multisubstrate inhibitors of tyrosine-specific protein kinases are described. One of the substrates, ATP, was mimicked by the known kinase inhibitor 5′-[4-(fluorosulfonyl)benzoyl]adenosine, which was covalently linked via the sulfonyl moiety to tyrosine mimics. The resulting multisubstrate inhibitors were tested for their ability to inhibit the transfer of phosphate from ATP to a protein acceptor by $p60^{v\text{-}abl}$, the tyrosine kinase encoded by the transforming gene (*v-abl*) of the Abelson murine leukemia virus (A-MuLV). Although the series of inhibitors displayed moderately potent activity (IC_{50} values as low as 19 μM), the absence of large effects produced by modification of the tyrosine mimic suggests that they do not behave as multisubstrate inhibitors but bind primarily through the adenosine moiety common to all the inhibitors. This interpretation is strengthened by the finding that the inhibitors lack specificity, inhibiting a serine kinase at comparable concentrations.

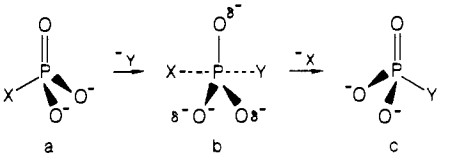
Recent studies on the molecular genetics of neoplastic transformation have identified a family of genes, designated oncogenes, whose aberrant expression has been implicated in the acquisition of tumorigenic properties.[1,2] Expression of closely homologous gene sequences by RNA tumor viruses[1] has also been found to be essential for the neoplastic conversion of infected cells. A number of oncogene-encoded proteins display tyrosine kinase activity and transfer the γ-phosphate of ATP to phenolic hydroxyl residues within protein substrates.[1,3] This specificity is unusual in that most protein kinases phosphorylate target substrates at serine or threonine residues.[4]

In normal cells, several cell surface growth factor receptors (e.g. PDGF,[5-7] EGF,[8] TGF-α, insulin,[9,10] and somatomedin C[11,12]) display tyrosine kinase activity. These receptors are considered to comprise a hydrophilic external domain that binds the growth factor(s), a transmembrane sequence and a catalytic domain that resides on the cytoplasmic face of the plasma membrane. Binding of growth factor activates the receptor kinase[13] to undergo autophosphorylation and to phosphorylate closely adjacent molecules.[14] The physiological significance of this reaction is not established, but it is considered to play an important role in signal transduction whereby extracellular mitogenic signals activate the intracellular pathways involved in cell proliferation.[15-17]

These observations have prompted the speculation that the principal function of tyrosine kinase activity in normal cells is to regulate cell growth. Perturbation of this activity by oncogene-encoded tyrosine kinases that are either overproduced and/or display altered substrate specificity relative to their normal cellular counterparts may cause loss of growth control and/or neoplastic transformation. Experimental evaluation of this concept requires the availability of inhibitors of oncogene tyrosine kinase activity. The present study describes the design of a series of novel tyrosine kinase inhibitors.

Experimental Strategy. Kinases or phosphotransferases catalyze the transfer of the γ-phosphate of nucleoside triphosphates (usually ATP) to a functional group on an acceptor molecule. X-ray crystallography and NMR analysis have been particularly useful in helping to define the nature of the binding sites for the two substrates. Kinetic studies and the use of ATP analogues bearing chiral γ-phosphate groups have revealed that many kinases do not utilize a mechanism involving a phosphorylated enzyme intermediate.[18] Instead, the enzyme serves as a template to align the substrates so that direct nucleophilic attack by the acceptor functional group on the γ-phosphate is facilitated. The transition state or midpoint of the reaction coordinate can be visualized as b shown in Scheme I.

Scheme I



While there is evidence to support this mechanism, there is debate about how closely the process resembles an S_N2 reaction, associative, or a dissociative reaction in which the transitory phosphorus is enzyme-bound metaphosphate.[19,20] In principle, these alternatives can be distinguished, but experimental evidence is ambiguous. In the

† Department of Medicinal Chemistry.
‡ Department of Cell Biology.

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(16) Downward, J.; Parker, P.; Waterfield, M. D. *Nature (London)* **1984**, *311*, 483.
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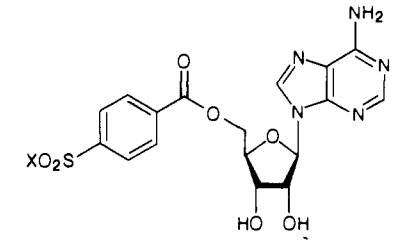
case of both hexose kinase and cAMP-dependent protein kinase the γ-phosphorus of ATP appears to be located too far from the attacking nucleophile to permit direct binding (6 and 5.3 Å, respectively).[21,22] These data were derived from X-ray or NMR measurements on the resting enzyme with substrates or products, and consequently it can be argued that the intersubstrate distances may decrease as the transition state is approached. It is important to realize that the substrate binding sites may be more distant than that predicted by a strict S_N2 analogy.

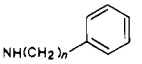
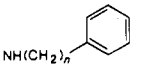
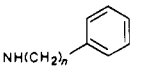
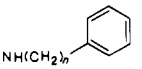
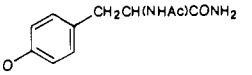
The lack of racemization of the transferred phosphate could arise from greatly restricted motion of the enzyme-bound metaphosphate intermediate. The observation that a combination of ADP, a planar ion (nitrate), and acceptor (creatine) form a dead-end complex that strongly inhibits creatine kinase could be used to support either mechansim.[14,23] In attempting to design kinase inhibitors, precise distinction between the associative and dissociative mechanisms may not be critical.

The approach we adopted was to synthesize transition state or multisubstrate analogues as inhibitors of tyrosine-specific protein kinases. Multisubstrate inhibitors contain covalently linked elements of both substrates as they exist during the catalytic event. This class of compounds frequently displays high and unique affinities for their enzyme targets. Even when the enzyme–substrate complex is imperfectly mimicked, occupancy of two binding sites by a single molecular species offers a substantial gain in binding energy. Application of this strategy is only appropriate with kinases that directly phosphorylate their substrates without formation of a phosphorylated enzyme intermediate. On the basis of studies with other classes of kinases,[24] and more recent investigations by Goldberg and Wong[25] on $p60^{v\text{-}src}$, it is reasonable to assume that tyrosine phorphorylation of angiotensin II by this kinase proceeds via a ternary complex, not a phosphorylated enzyme intermediate, and thus would be amenable to inhibition by multisubstrate analogues.

Structural elements of ATP can be readily incorporated into multisubstrate inhibitors, but the structural requirements for the tyrosine binding site remain unestablished. The endogenous substrates for tyrosine kinases are not known.[26] Most studies on isolated kinases have used casein or antikinase IgG as substrates. The autophosphorylation site of $p60^{v\text{-}src}$, the tyrosine kinase encoded by Rous sarcoma virus,[27] is probably a more physiologically relevant substrate as canges at tyrosine-416 in this molecule alter the oncogenic potential of the virus. The amino acid sequence flanking the tyrosine-416 residue has been determined,[28] and peptide analogues have been synthesized and tested as substrates.[29-33] Initially, these studies

Table I. Inhibition of $p60^{v\text{-}abl}$ Tyrosine Kinase Activity[a]



compd	X	n	IC_{50}, μM
1	F		150
3	NH_2		120
4		0	19
5		1	45
6		2	50
7		3	56
8			170
9			120
10	CH_3		63

[a] Methods and calculation of IC_{50} values are described in the Experimental Section.

suggested that acidic residues located in the peptide amino terminus were essential in enzyme–substrate recognition and binding because this structural feature was shared by a number of $p60^{v\text{-}src}$ substrates including the autophosphorylation site of $p60^{v\text{-}src}$, $p90^{v\text{-}yes}$,[28] peptide analogues of polyoma middle T antigen,[29] and gastrin.[34] More recently, it has been shown that angiotensin and related analogues that lack the cluster of acidic amino acids[28-33] are also phosphorylated with approximately the same efficiency. The peptides belonging to both classes are usually poor substrates and display K_m values in the low millimolar range.[35] This indicates that the precise structural determinants responsible for substrate recognition and phosphorylation have yet to be identified and probably involve higher orders of protein structure. Even in the absence of this information it is reasonable to suggest that the aromatic ring and phenolic hydroxyl of target tyrosine residues are key elements in substrate recognition. The absolute specificity of tyrosine kinases for this residue as opposed to serine and threonine support this contention. On the basis of this assumption, one chemical approach has been to construct multisubstrate inhibitors containing structural elements of ATP covalently linked to simple aromatic residues or tyrosine derivatives with the expectation that affinity could be largely provided by binding at the ATP site while selectivity would derive from the portion of the inhibitor that binds to the tyrosine site.

Because tyrosine kinases are principally intracellular,[36] an important part of the present strategy has been to

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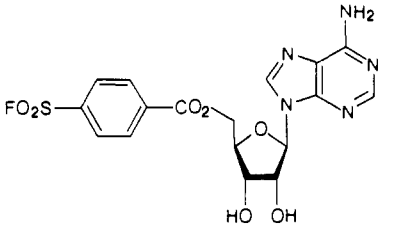
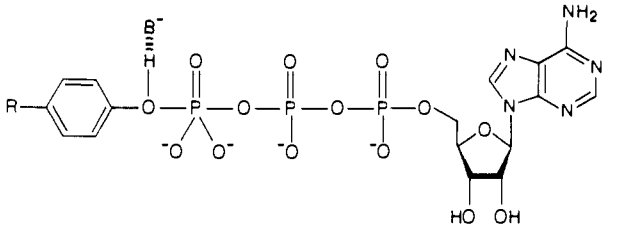
design membrane-permeable inhibitors. This necessitates replacement of the highly charged triphosphate portion of the multisubstrate analogues with a more hydrophobic moiety. Initially we selected the known kinase inhibitor, 5′-[4-(fluorosulfonyl)benzoyl]adenosine or 5′-FSBA (**1**),



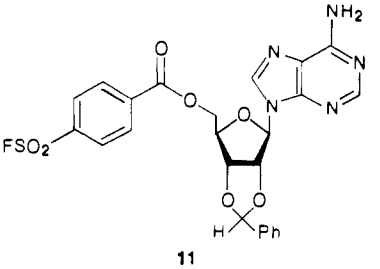
1 (5′-FSBA)

which selectively forms covalent adducts with enzyme ATP binding sites and inhibits phosphorylation of serine and threonine residues.[37] The carbonyl and sulfonyl groups may be capable of mimicking the α- and γ-phosphates of ATP while the fluorosulfonyl functionality offers a possible attachment site for tyrosine mimics. 5′-FSBA has been also shown to label lysine residues specifically at the active site of $p60^{v\text{-}src}$ and the epidermal growth factor (tyrosine kinase) receptor.[38,39] Here we report the synthesis and testing of a series of potential tyrosine protein kinase inhibitors (**3–10**, Table I) designed to probe the distance between the ATP and tyrosine binding sites and to mimic the proposed intermediate of the phosphate transfer reaction (**2**).



2

Synthesis of Inhibitors. Compound **1** was synthesized according to Colman's procedure.[40] Attempts to use this compound directly to construct the desired target sulfonamido inhibitors proved unsuccessful due to the lack of reactivity of the fluorosulfonyl moiety to displacement. Compounds **8** and **9**, however, could be elaborated from 2′,3′-benzylidene-5′-[*p*-(fluorosulfonyl)benzoyl]adenosine (**11**) by reaction with the sodium salt of the requisite phenols in DMF containing the acylation catalyst 4-(dimethylamino)pyridine (DMAP). Deprotection with 80% aqueous acetic acid followed by chromatography afforded the desired sulfonic esters.



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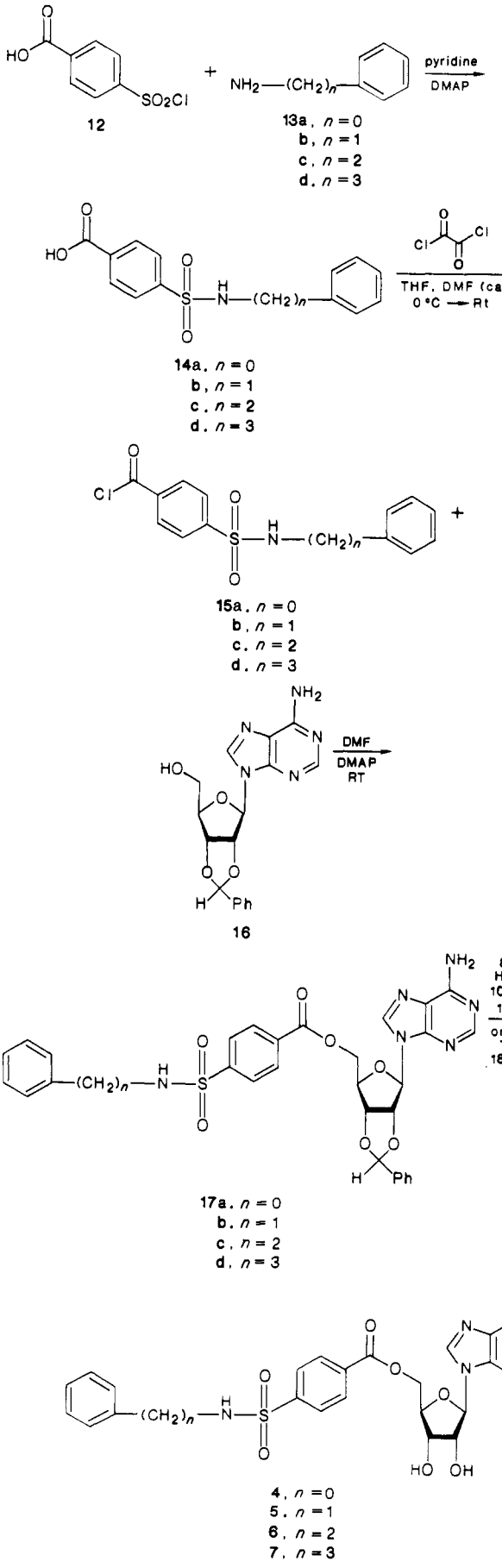
Scheme II. Preparation of Sulfonamide Derivatives



(37) Colman, R. F. *Annu. Rev. Biochem.* **1983**, *52*, 67.
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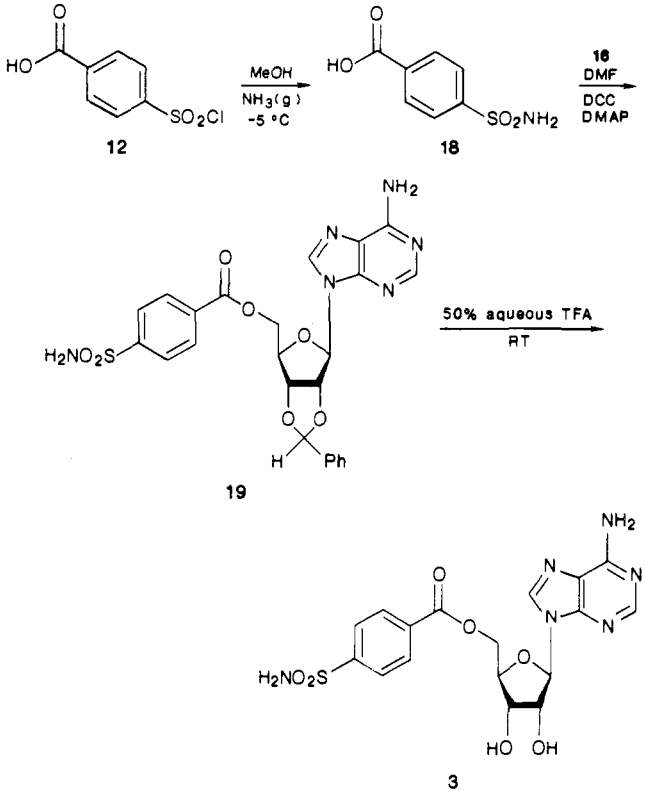
(40) Colman, R. F.; Pal, P. K.; Wyatt, J. L. *Methods Enzymol.* **1977**, *46*, 240.

Scheme III. Preparation of Primary Sulfonamide 3



Sulfonamides **4–7** were prepared by the general method shown in Scheme II. A concentrated solution of the appropriate aromatic amine (**13a–d**) in pyridine, containing DMAP, was treated with *p*-(chlorosulfonyl)benzoic acid (**12**) by addition of the solid in small portions. This method of addition was necessary to avoid the formation of a mixed anhydride, which gave rise to substantial amounts of a byproduct resulting from the addition of the amines to both the acid and to the sulfonyl chloride functionalities. Formation of the benzoyl chlorides **15a–d** with oxalyl chloride was followed by addition of 2′,3′-benzylideneadenosine (**16**)[41] to give the benzylidene protected sulfonamides **17a–d**. The benzylidene protecting group was removed with 80% aqueous acetic acid or by treatment with 50% aqueous TFA at room temperature to give sulfonamides **4–7**.

The unsubstituted sulfonamide **3** was synthesized from sulfonamide **18** as indicated in Scheme III. Addition of *p*-(chlorosulfonyl)benzoic acid (**12**) to a concentrated solution of ammonia in methanol gave **18**. Problems encountered in attempting to form the adenosine adduct **19** through an acid chloride were circumvented by using DCC coupling. Deblocking with 50% TFA gave **3**.

The *p*-methylsulfonyl derivative, **10**, was prepared via formation of the acid chloride from commercially available *p*-(methylsulfonyl)benzoic acid followed by addition of the benzylidene protected adenosine **16** and deprotection by treatment with 50% TFA. In all cases the final products were puridied by flash chromatography[42] using silica gel with 10% MeOH–$CHCl_3$ as eluant.

Enzyme Assays. Tyrosine Kinase Assay. The transforming gene (*v-abl*) of the Abelson murine leukemia virus (A-MuLV) was used as a source of tyrosine kinase activity. This ezyme can be isolated from A-MuLV infected cells but in very poor yield. To provide an adequate supply of enzyme we have cloned the *v-abl* gene into an *Escherichia coli* expression system and purified the enzyme as described previously.[43] The recombinant *v-abl* product, $p60^{v\text{-}abl}$, retained full enzymatic activity. Compounds were tested for their ability to inhibit the phosphorylation of tyrosine residues by $p60^{v\text{-}abl}$ with α-casein as substrate. Assay conditions are described in the Experimental Section.

Time Course of Inhibition. Previous work had established[44] that the incorporation of ^{32}P from radiolabeled γ-ATP into α-casein catalyzed by p-$60^{v\text{-}abl}$ is linear with time under the conditions of this assay. The inhibition of this reaction by 5′-FSBA and two representative compounds, **5** and **9**, was tested after preincubation of inhibitors for various intervals with the enzyme. In some experiments the preincubation time was increased up to 60 min. Otherwise the assay conditions were identical.

Serine Kinase Assay. To check the specificity of the inhibitory effect of test agents, compounds were evaluated for their ability to block serine-specific phosphorylation of phosphorylase by phosphorylase kinase. Conditions are described in the Experimental Section.

Results and Discussion

An important initial objective of the present study was to examine the distance between the ATP and tyrosine binding sites to establish optimal inhibitor dimensions and to better define the requirements of the tyrosine binding site for rational design of potent, specific inhibitors capable of crossing the cell membrane and binding strongly or irreversibly to the active site of tyrosine-specific protein kinases. The multisubstrate inhibitor approach utilized here assumed that both the tyrosine and ATP substrates bind to the enzyme in a ternary complex with a small intersubstrate distance. The present data cannot preclude an ordered binding mechanism involving a phosphorylated enzyme intermediate, though this is viewed as unlikely based on the kinetic studies of Goldberg and Wong[25] on the related tyrosine kinase, $p60^{v\text{-}src}$.

Sulfonamides **4–7** were synthesized to probe the distance between the adenosine and tyrosine binding sites and to examine the necessity of the acidic NH for recognition. The IC_{50} data suggest that an acidic NH, which may function as a mimic of the phenolic hydrogen of the tyrosine substrate, increases the potency of this series of inhibitors (See **4** vs **8**, Table I), supporting our assumption that the OH might be important in enzyme recognition. In contrast, sulfone **10** is more potent than **3**. Varying the chain length between the NH and aromatic ring had little effect on inhibitory potency of the compounds due either to a lack of recognition of the tyrosine mimics or a less than optimal intersubstrate distance. The similarity of the IC_{50} values for compounds **3** through **7** suggests that these agents bind only at the ATP site and that additional affinity is not achieved by incorporation of aromatic substituents. This conclusion is supported by the decreased potency of the phenol and blocked tyrosine derivatives (**8** and **9**) relative to the sulfonamides and the subsequent finding that neither phenol nor *N*-acetyltyrosinamide served as an enzyme substrate.

5′-FSBA showed time-dependent inhibition of the enzyme with almost complete inactivation at 60 min (Figure 1), suggestive of covalent binding of this derivative. As

(41) Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* 1949, 2476.
(42) This and subsequent references to "flash chromatography" refer to the technique developed by Still. Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978**, *43*, 2923.
(43) Ferguson, B.; Pritchard, M. L.; Feild, J.; Rieman, D.; Greig, R.; Poste, G.; Rosenberg, M. *J. Biol. Chem.* **1985**, *260*, 3652.
(44) Maness, P. F.; Perry, M. E.; Levy, B. T. *J. Biol. Chem.* **1983**, *258*, 4055.

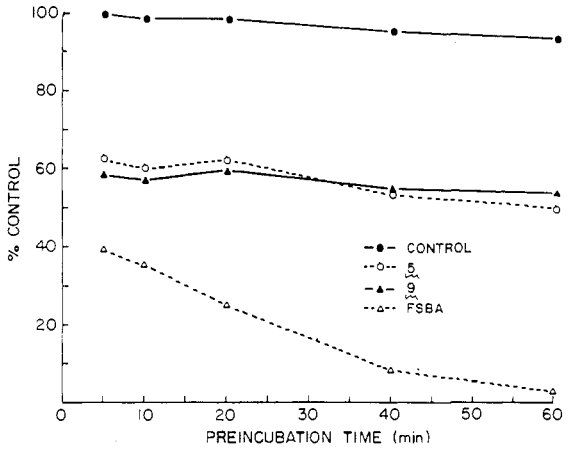
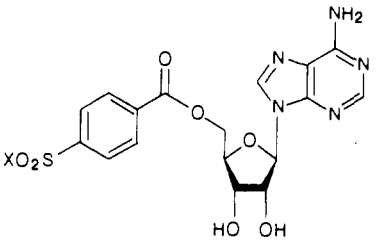
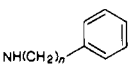


Figure 1. Inhibition of $p60^{v\text{-}abl}$ tyrosine kinase activity: effect of preincubation time. Compounds were assayed in duplicate; values were within 5%.

Table II. Inhibition of Phosphorylase (Serine) Kinase Activity[a]



compd	X	n	IC_{50}, μM
1	F		250
3	NH_2		60
4		0	25
7		3	50
8			80

[a] Methods and calculation of IC_{50} values are described in the Experimental Section.

expected, neither **5** nor **9**, the benzylamine and *N*-acetyltyrosinamide derivatives, displayed any change in inhibitory activity as a function of preincubation time. The sulfonamides displayed similar inhibition patterns for the serine kinase, phosphorylase kinase, supporting the conclusion that the compounds bind primarily at the ATP site. A similar conclusion was reached by Maness in studying the inhibition of $p60^{v\text{-}src}$ by P^1,P^4-diadenosine-5′ tetraphosphate in the presence of increasing ATP concentrations.[44] The similar IC_{50} values for these compounds in inhibiting phosphorylation of tyrosine and serine (Table II) indicates that they lack the desired specificity and that binding to the enzymes occurs primarily through the adenosine portion of the inhibitors. Both classes of kinases probably require not only the target amino acid residue but also other structural features for efficient substrate recognition. Primary sequences and higher orders of protein structure may be important determinants of recognition.

These results indicate that more information is required on the tyrosine binding site before more potent inhibitors can be designed. We are currently evaluating a series of small peptides as substrates of $p60^{v\text{-}abl}$. The participation of the triphosphate bridge, replaced in the present studies with a *p*-(fluorosulfonyl)benzoyl moiety, in substrate recognition must also be considered. To explore this possibility, compounds containing tri- and tetraphosphate bridges have been tested as $p60^{v\text{-}abl}$ inhibitors (see the accompanying paper).

Experimental Section

Materials and Methods. Solvents were dried and/or purified by standard procedures.[45] Thin-layer chromatography was done on Analtech Uniplate 250 μm silica gel plates. Baker 40 μm flash silica gel was used for flash chromatography. Compounds were characterized by NMR, IR, and MS analyses. IR spectra were recorded on a Perkin-Elmer 783 spectrophotometer as Nujol mulls. NMR spectra were obtained as $CDCl_3$ solutions on a Varian EM390 spectrometer, and chemical shifts were recorded relative to tetramethylsilane. Chemical ionization mass spectra were obtained with a Finnigan 3300/9500 mass spectrometer. Field desorption mass spectra were obtained with a Varian MAT DF mass spectrometer. Fast atom bombardment mass spectra were obtained with a VG ZAB IF-HF spectrometer equipped with a standard FAB ion source with a glycerol matrix. Elemental analyses for C, H, and N were performed by the Analytical, Physical and Structural Chemistry Department of Smith Kline & French Laboratories. Analytical values were within 0.4% of the calculated values, except where noted.

Carrier-free [^{32}P]orthophosphoric acid was purchased from Amersham Corp. [γ-^{32}P]ATP was prepared by using the Gamma Prep System from Promega Biotec, and product purity was checked by thin-layer chromatography with PEI cellulose plates purchased from E. Merck Co., eluted with 1.6 M LiCl. Protein was determined by Bradford's method[46] with bovine serum albumin as a standard. Octylglucopyranoside and dithiothreitol were obtained from Calbiochem Behring. Coomassie G250 stain was purchased from Serva Fine Chemicals. Other reagents used in enzyme isolation and activity assays were purchased from either Fisher Chemical Co., Sigma Chemical Co., or J. T. Baker. Radioactivity was measured with a Beckman LS2800 Liquid Scintillation Counter. The Mono Q column and FPLC system were purchased from Pharmacia.

Isolation of Abelson Tyrosine Kinase. The enzyme $p60^{v\text{-}abl}$ was extracted from *E. coli* as described.[43] $p60^{v\text{-}abl}$ remained insoluble until the final step facilitating the removal of bacterial proteins by sequential treatment with lysozyme, sodium deoxycholate, and octylglucoside. Following solubilization in 2 M KSCN in buffer (50 mM Tris·HCl, pH 8.0, 2 mM EDTA, 2 mM DTT) $p60^{v\text{-}abl}$ was desalted by dialysis against 50 mM Tris·HCl, pH 7.5, 1 mM EDTA, and 0.1 mM DTT. The dialyzed material was then applied to a Mono Q FPLC column system equilibrated with buffer (50 mM Tris·HCl, pH 7.5, 1 mM EDTA, and 0.1 mM DTT) and eluted with a 10-mL linear KCl gradient (0–0.5 M) in that buffer. Peak activity eluting at 100–150 mM KCl was pooled, glycerol was added to a final concentration of 50%, and the enzyme was stored at −20 °C. The final product was ca. 70% pure, on the basis of Coomassie blue staining of SDS-PAGE gels.[43]

Assay of Tyrosine Protein Kinase Activity. Enzyme activity was measured with α-casein as substrate. Inhibitors were preincubated with the enzyme for 5 min at 25 °C. Reaction conditions were 100 mM MOPS, pH 7.0, 10 mM $MgCl_2$, 2 μm [γ-^{32}P]ATP (6 Ci/mmol), 1 mg/mL casein and 7.5 μg/mL of enzyme in a total volume of 30 μL. The reactions were incubated for 10 min at 25 °C. Trichloroacetic acid precipitation of protein was followed by rapid filtration[43] and quantification of phosphorylated casein by a liquid scintillation counter. Compounds were tested in quadruplicate at four concentrations (1×10^{-5} M, 5×10^{15} M, 1×10^{-4} M, and 5×10^{-4} M).

Time Course Assay of Inhibition. The time course assay of tyrosine protein kinase activity was conducted as described above except that ^{32}P incorporation was measured after preincubation of enzyme at 5, 10, 20, 40, and 60 min as indicated in Figure 1. Compounds were tested at a concentration that gave 60–80% inhibition after 5 min of preincubation.

Assay of Serine Kinase Activity. Serine kinase activity of phosphorylase kinase was measured in an assay similar to the tyrosine kinase assay with phosphorylase b as substrate. Reaction

(45) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966.
(46) Bradford, M. M. *Anal. Biochem.* 1976, 72, 248.

conditions were 100 mM Tris·HCl, pH 7.1, 10 mM $MgCl_2$, 2 μM [γ-^{32}P]ATP (6 Ci/mmol), 1 unit/mL heparin, 1.7 mg/mL phosphorylase b and 13 μg/mL phosphorylase kinase.

5′-(4-Sulfamoylbenzoyl)adenosine (3). To a concentrated solution of ammonia gas in dry MeOH (50 mL) at −5 °C was added 4-(chlorosulfonyl)benzoic acid (**12**) (1.5 g, 6.8 mmol) in small portions. After addition, TLC (2:7.5:0.5 methanol–chloroform–HOAc) showed no starting material remaining. The reaction mixture was concentrated and flash chromatographed with 1.5:7:1 methanol–methylene chloride–HOAc to give the desired sulfonamide (**18**) (725 mg, 53%) as a white powder. FD MS, IR, and NMr analyses were consistent with the desired product. The sulfonamide acid **18** and **16** were dissolved in dry DMF (25 mL) containing a catalytic amount of DMAP. The solution was cooled to 0 °C and treated with DCC (1 equiv). After being warmed to room temperature, the reaction mixture was allowed to stir overnight. The mixture was filtered, concentrated, and flash chromatographed on silica gel with 3% methanol–chloroform to give the desired benzylidene protected product (**19**). The product was deprotected with 50% aqueous TFA at room temperature. Flash chromatography on silica with 10% methanol–chloroform gave **3** as the TFA salt: (29% yield) MS-FAB, *m/e* 451 (M^+ + H); NMR (DMSO-d_2) δ 7.4–7.7 (m, 6 H), 4.05–4.45 (m, 5 H), 5.55 (d, *J* = 3 Hz, 1 H). Anal. ($C_{17}H_{18}N_6O_7S·C_2HO_2F_3$) C, H, N.

5′-[4-(Anilinosulfonyl)benzoyl]adenosine (4). To a 50% solution of aniline in dry pyridine (5 mL) at room temperature under argon was added *p*-(chlorosulfonyl)benzoic acid (0.25 equiv) in small portions. After 29 h the reaction mixture was diluted with EtOAc and washed with cold 1 N HCl and brine, dried, and concentrated to leave a yellow oil. Flash chromatography on silica with EtOAc gave the adduct (**14a**) as a clear oil. The acid was treated with 5 equiv of oxalyl chloride in THF containing a catalytic amount of DMF at 0 °C under argon. After being warmed to room temperature, the reaction mixture was stirred for 2 h, and then the mixture was concentrated in vacuo and used without purification in the next reaction. To a solution of **16** in dry DMF containing a catalytic amount of DMAP at room temperature under argon was added dropwise a solution of the acid chloride (1 equiv) in DMF. After 2 h the solution was concentrated in vacuo to leave a brown oil, which was flash chromatographed on silia gel eluted with 10% hexane–ethyl acetate to give the desired ester **17a** as a white solid. The material was deblocked by treatment with 80% aqueous HOAc at 100 °C for 18 h. Flash chromatography on silica eluted with 10% methanol–chloroform afforded **4** (34% yield) as a white solid: MS-FD, *m/e* 527 (M^+ + H); NMR (acetone-d_6) δ 7.3–7.8 (m, 6 H), 6.85 (m, 5 H), 6.2 (brs, 1 H), 5.6 (d, *J* = 4 Hz, 1 H), 4.0–4.5 (m, 5 H). Anal. ($C_{23}H_{22}N_6O_7S·H_2O$) C, H, N.

Sulfonamides **5** (17% yield), **6** (26% yield), and **7** (47% yield) were prepared in a similar manner. **5**: MS-FAB, *m/e* 541 (M^+ + H); NMR (acetone-d_6) δ 7.4–7.8 (m, 6 H), 6.7 (brs, 1 H), 5.4 (d, *J* = 4 Hz, 1 H), 4.5–4.0 (m, 3 H), 3.5–3.8 (m, 3 H). Anal. ($C_{24}H_{24}N_6O_2S$) C, H, N. **6**: MS-FD, *m/e* 555 (M^+ + H); NMR (D_2O) δ 7.3–71.8 (m, 6 H), 6.4–6.8 (m, 5 H), 5.35 (d, *J* = 4 Hz, 1 H), 4.0–4.6 (m, 5 H) 3.2–3.4 (m, 2 H), 2.5–2.9 (m, 2 H). Anal. ($C_{25}H_{26}N_6O_7S·0.75H_2O$) C, H, N. **7**: MS-FAB, *m/e* 469 (M^+ + H); NMR (D_2O) δ 7.6–8.2 (m, 6 H), 6.8–7.0 (m, 5 H), 5.6 (d, *J* = 4.5 Hz, 1 H), 4.1–4.6 (m, 5 H) 3.9–4.05 (m, 2 H), 3.7 (t, *J* = 4 Hz, 2 H), 3.0 (m, 2 H). Anal. ($C_{26}H_{28}N_6O_7S$) C, H, N.

5′-[4-(Phenoxysulfonyl)benzoyl]adenosine (8). A solution of phenol (26.3 mg, 0.28 mmol) in DMF (5 mL) was treated with NaH (1 equiv) and allowed to stir for 15 min at room temperature under argon. The solution was treated with 1 equiv of **11** added as a solid all at once. After the mixture was stirred for 18 h, TLC (10% methanol–chloroform) showed one new spot with an R_f almost identical with that of the starting material (0.75). The material was flash chromatographed with a methanol–chloroform gradient to leave a clear oil, shown by NMR, IR and FD MS analyses to be the desired product. The material was deprotected with 50% aqueous TFA at room temperature. Flash chromatography with 10% methanol–chloroform afforded the desired product, **8** (66% yield): MS-FAB, *m/e* 528 (M^+ + H); NMR (acetone-d_6) δ 7.4–8.0 (m, 6 H), 7.05–6.5 (m, 5 H), 5.66 (e, *J* = 4.5 Hz, 1 H), 4.0–5.6 (m, 5 H). Anal. ($C_{23}H_{21}N_5O_8S$) C, H, N.

Compound **9** was prepared in a similar manner (32% yield) except that it was deprotected with 80% aqueous HOAc at 100 °C: MS-FD, *m/e* 656 (M^+ + H); NMR (acetone-d_6) δ 8.0–7.3 (m, 6 H), 7.0–6.5 (q, 4 H), 5.7 (d, *J* = 4.5 Hz, 1 H), 3.7–4.8 (m, 8 H), 2.85 (m, 2 H). Anal. ($C_{28}H_{29}N_7O_{10}S·1.75H_2O$) C, H; N: calcd, 14.27; found, 13.69.

5′-[4-(Methylsulfonyl)benzoyl]adenosine (10). A stirred suspension of 4-(methylsulfonyl)benzoic acid (0.5 g, 2.5 mmol) in dry THF (20 mL) containing 3 drops of DMF was treated with oxalyl chloride (2 equiv) at room temperature under argon. After the mixture was stirred for 48 h, the acid had dissolved completely and IR showed complete conversion to the acid chloride. The reaction mixture was concentrated to remove excess oxalyl chloride and redissolved in 5 mL of THF. This solution was added dropwise to a solution of **16** (0.9g, 2.5 mmol) in DMF (20 mL) containing a catalytic amount of DMAP at room temperature under argon. After the mixture was stirred overnight, TLC (10% hexane–ethyl acetate) showed one major, new spot. The reaction mixture was concentrated and then flash chromatographed on silica with a hexane–ethyl acetate gradient to leave the desired ester as a white foam. The compound was deblocked with 50% aqueous TFA at room temperature for 4 h. Flash chromatography on silica gel with 10% methanol–chloroform afforded the TFA salt of **10** (50% yield) as a white powder: MS-FAB, *m/e* 450 (M^+ + H); NMR (D_2O) δ 7.4–7.8 (m, 6 H), 5.55 (d, *J* = 4 Hz, 1 H), 2.95 (s, 3 H). Anal. ($C_{18}H_{19}N_5O_7S·C_2HO_2F_3$) C, H, N.

2′,3′-Benzylidene-5′-[4-(fluorosulfonyl)benzoyl]adenosine (11). To a stirred solution of **16** (150 mg, 0.42 mmol) in dry DMF (3 mL) containing DMAP (52 mg, 1 equiv) was added 1 equiv of 4-(fluorosulfonyl)benzoyl chloride, which had been freshly recrystallized from petroleum ether. After 1 h the reaction was concentrated and flash chromatographed on silica with ethyl acetate as eluant to give **11** as a white solid (230 mg, ca. 100%). CI MS, IR, and NMR analyses were consistent with the proposed structure. Anal. ($C_{24}H_{20}N_5O_7SF$) C, H, N.

Registry No. 1, 57454-44-1; **3**, 114958-08-6; **3**·TFA, 114958-18-8; **4**, 114958-09-7; **5**, 114958-10-0; **6**, 114958-11-1; **7**, 114958-12-2; **8**, 114958-13-3; **9**, 114958-14-4; **10**, 114958-15-5; **10**·TFA, 114958-29-1; **11**, 114958-16-6; **12**, 10130-89-9; **13a**, 62-53-3; **13b**, 100-46-9; **13c**, 64-04-0; **13d**, 2038-57-5; **14a**, 6314-72-3; **14b**, 10252-76-3; **14c**, 114958-23-5; **14d**, 114958-26-8; **15a**, 114958-19-9; **15b**, 114958-21-3; **15c**, 114958-24-6; **15d**, 114958-27-9; **16**, 3257-69-0; **17a**, 114958-20-2; **17b**, 114958-22-4; **17c**, 114958-25-7; **17d**, 114958-28-0; **18**, 138-41-0; **19**, 114958-17-7; PhOH, 108-95-2; $MeSO_2C_6H_4$-*p*-COOH, 4052-30-6; $MeSO_2C_6H_4$-*p*-COCl, 40913-92-6; $FSO_2C_6H_4$-*p*-COCl, 402-55-1; tyrosine-specific protein kinase, 80449-02-1; phosphorylase (serine) kinase, 9031-44-1.